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THE USE OF VIRUS TO CONTROL TUSSOCK MOTH

PROGRESS REPORT

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THE USE OF VIRUS TO CONTROL TUSSOCK MOTH

SUMMARY

Supplies of virus can be maintained or increased in the laboratory by spraying immature tussock moth larvae with a virus solution at the rate of 1 million polyhedra per ml., 1/12 ml. per cage; optimum virus concentration tested was 10 million polyhedra per ml., 1/12 ml. per cage.

Infected immature larvae yielded approximately 50 million, and mature larvae yielded about 300 million polyhedra per individual.

Virus was established when applied at the rate of 10 million polyhedra per acre against a tussock moth infestation on bitterbrush. Excellent control can be obtained the same year with an application of 50 million polyhedra per acre.

Corn syrup (10 percent by volume) proved to be a better sticking agent than powdered milk.

The tussock moth population in all treated blocks had practically disappeared the year following treatment.

INTRODUCTION

Records show that many epidemics of tussock moths have been reduced to insignificant levels by the action of native viruses. Unfortunately, viruses are not always present or, if present, do not always increase fast enough to reduce populations before damage exceeds a tolerable level. Therefore, a method of artificially infecting a tussock moth outbreak with virus could be a valuable control tool. Knowledge of field application and concentrations that might be effective against tussock moth was limited, so some laboratory work was necessary before proceeding with tests. The objectives of this work were to determine: (1) the host specificity of a virus collected in Owyhee County, Idaho, (2) the minimum virus concentration needed to control a tussock moth infestation, (3) the most efficient rearing method and virus concentration needed to produce a stock supply, and (4) a practical formulation for field application of the virus.

The virus strain used in these experiments was obtained from the Douglas-fir tussock moth infestation on Douglas-fir and true firs in Owyhee County, Idaho. This was the second time in ten years that an epidemic had occurred and had been controlled in Owyhee County by a native virus[1/], [2/], [3/], [4/].

1/ Orr, L. W. 1951. Present status of Douglas-fir tussock moth outbreak in Owyhee County, Idaho. Mimeographed.

2/ Cole, W. E. 1956. Douglas-fir tussock moth infestation in Owyhee County, southern Idaho. Correspondence to the files.

3/ Cole, W. E. 1957. Douglas-fir tussock moth appraisal survey Owyhee County, Idaho. Mimeographed.

4/ Cole, W. E. 1958. Douglas-fir tussock moth situation in Owyhee County, Idaho. Mimeographed.

Coincident with this tussock moth infestation others were reported on white fir in New Mexico, and on bitterbrush near Carson City, Nevada[5/]. Consequently, the virus was tested in the laboratory against tussock moth from the three localities. However, field tests were made only on the tussock moth infesting bitterbrush.

LABORATORY METHODS

The isolated, controlled temperature room of an insectary at the Research Center at Boise, Idaho, was used for inoculation tests. In order to maintain as nearly aseptic conditions as possible, the room was swabbed once a week with a 1 to 9 Clorox solution. Similar tests were conducted at Ogden, Utah, where inoculations were accomplished in the laboratory and sterile check larvae were reared in the greenhouse. The plastic rearing cage technique[6/] was used and no cage used more than once (Photo 1).

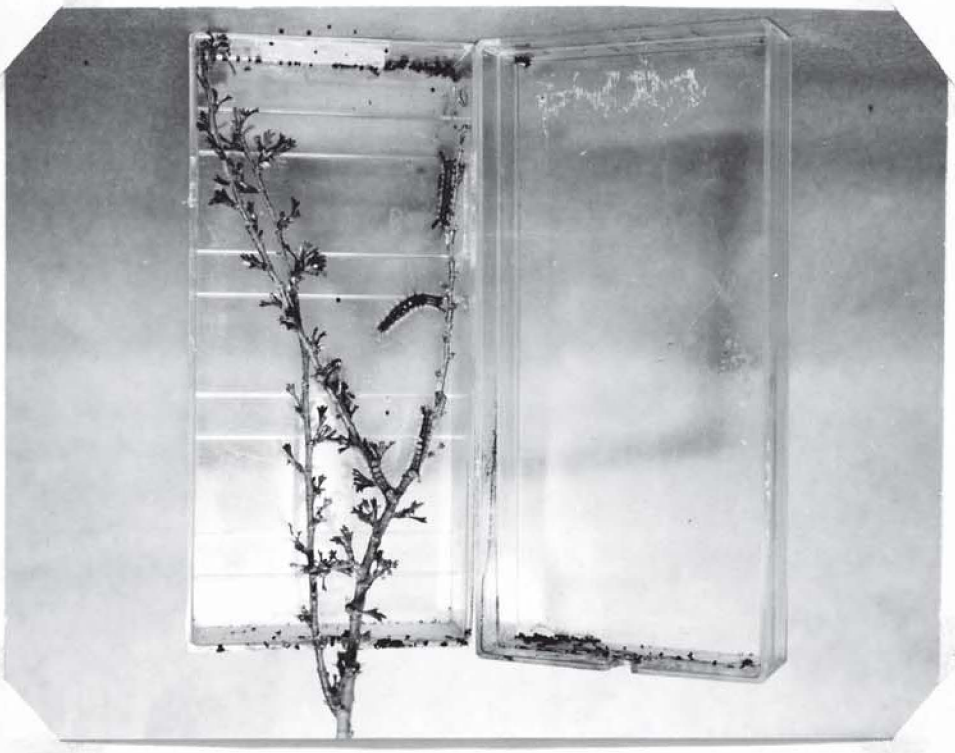

Photo 1. Plastic rearing cage used in inoculation test, showing host plant and tussock moth.

5/ Washburn, R. I. 1958. Tussock moth infesting range plants, Carson City, Nevada, appraisal survey. Mimeographed report.

6/ Randall, A. P. 1957. Plastic rearing cage for maintaining fresh conifer foliage for insect rearing. Canad. Ent. 89(10): 448-449.

In the Boise rearings, 15 larvae were placed within each cage, and in the Ogden rearings, 5 larvae were placed within each cage. Foliage was sprayed with each virus concentration with a calibrated atomizer prior to placement in the cage (photos 2 & 3). A haemacytometer was used for counting polyhedral bodies. One milliliter of distilled water was used with each virus-killed larva to standardize dilutions and obtain comparable recovery data.

Photo 2. Application of virus concentration to bitterbrush foliage for laboratory inoculation test.

Photo 3. Preparation of virus solution for inoculation of food supply prior to caging tussock moth larvae. (Note cages and moisture tray).

LABORATORY RESULTS

Several virus solutions, ranging from 1 to 82 million polyhedra per milliliter, were used in the tests. Formulations were based on 1 milliliter of the virus concentration to be used per gallon of distilled water. This solution was applied in the laboratory with an atomizer at a rate equivalent to 1 gallon of spray per acre in the field. Table 1 shows the results of these tests.

Table 1.--Percent mortality obtained in laboratory tests to determine virus concentrations for field use. Expressed in percent mortality.

Test location	Dosage (polyhedra per acre)	Source of larvae and host plant		
		Nevada bitterbrush	Idaho Douglas-fir	New Mexico white fir
Ogden	1.0×10^6	47	No tests conducted	
	2.3×10^6	59		
	5.2×10^6	51		
	check 1	28		
	check 2	13		
Boise	10×10^6	100	100	95
	20.5×10^6	100	100	92
	41.0×10^6	100	100	100
	61.5×10^6	100	100	100
	82.0×10^6	100	100	100
	check	100	50	44

The mortality obtained in laboratory tests clearly showed that tussock moth from Nevada and New Mexico were susceptible to the Idaho virus.

The high percent of mortality in the Boise checks, as compared with the Ogden checks (table 1) probably resulted from two causes: (1) The Boise tests utilized 15 larvae per cage; the Ogden tests 5 larvae per cage. Thus crowding could have been a factor in stimulating the latent virus, if the latent virus theory is applicable, and (2) more than likely rearing conditions were the most contributing factor to these differences. All of the Boise tests were conducted in a 6' x 10' room and even though care was taken to maintain the best aseptic condition possible, chances for contamination were high. The rearings in Ogden showed greater difference in survival between checks and tests where it was possible to use separate rooms for check and treated material.

Average immature infected larvae yielded approximately 50 million polyhedra per ml. when prepared at the rate of 2 larvae per 1 ml. of distilled water, and the average mature infected larva yielded approximately 300 million polyhedra per ml. when prepared at the rate of 1 larva per ml. of distilled water.

The yeast count method proved more reliable than the red blood corpuscle method for counting polyhedra per ml. concentration. The virus count was made from the concentration diluted 100 times in the pipette used for a red blood cell count by filling the capillary to the 1.0 mark with the virus concentration, and to the 101 mark with distilled water. The sample was shaken to give an even suspension and the counting chambers of the haemacytometer filled. Five groups of 16 small squares, which equals 1/250 cubic mm., were counted per chamber and averaged. The average number of polyhedra per group multiplied by 10^6 produces a count of polyhedra per milliliter.

FIELD METHODS

An experimental control test was conducted in the field during May 1959 when plots of infested bitterbrush in Nevada were sprayed. Three spray plots and 1 check plot, each one acre in size (2 x 5 chains) were established in a line, with 2-chain intervals between plots within each of two areas, Mt. Rose and Winnie Lane. These areas were separated by approximately 20 air miles. The spray was applied by a Buffalo turbine mist blower mounted on a power wagon that was driven down the road that bissected the test plots.

In view of the vast difference between laboratory and field conditions, it was decided that the virus concentration producing the highest mortality in the laboratory would be the lowest concentration used in the field tests. Three concentrations: ten million, fifty million, and one hundred million polyhedra per gallon, were applied at the rate of 1 gallon per acre. These concentrations were replicated by using 2 stickers, powdered milk and corn syrup, both as 10 percent by volume. The Winnie Lane area near Carson City, Nevada, received the powdered milk replicate, and the corn syrup dosage was applied to the Mt. Rose area near Reno, Nevada. The virus was applied when the majority of the larvae were in the second and third instars.

Prespray and postspray larval counts were made in all plots on 4, 15-inch twigs collected from each of 6 bushes per plot. The prespray counts were made the day before spraying. The postspray counts were made 6 weeks after spraying when the majority of the population was in the sixth instar and pupal stages.

FIELD RESULTS

Percent control, based on Abbott's formula, which allows for normal mortality, is shown in table 2.

Table 2.--Percent control of the tussock moth larvae on bitterbrush, with virus

Polyhedra/gallon (1 gallon/acre)	Sticking agent	
	Powdered milk	Corn syrup
10 million	not significant	63.41
50 million	59.33	90.23
100 million	87.89	not significant

With the 10 million concentration using powdered milk, mortality was not significant; while with corn syrup mortality was significant when compared with the mortality that occurred in the check plots.

The 50 million concentration, the corn syrup sticker replication gave higher mortality than with powdered milk.

Mortality caused by the 100 million concentration, as measured in the field, was not significant with the corn syrup, but was significant with the powdered milk formulation. However, mortality in the 50 million corn syrup test was approximately the same as the mortality in the 100 million, powdered milk test.

The test plot used for the 100 million, corn syrup application, proved to have a low population of larvae. This made sampling difficult and the mortality resulting from virus as determined by Abbott's formula proved not significant, probably because of larval population density. The tussock moth populations in each plot, based on averages of 4, 15-inch twigs from each of six bushes, are shown in table 3.

Table 3.--Prespray and postspray tussock moth larval populations on bitterbrush per 15-inch twig.

Area	Plot	Prespray population average	Postspray population average
Winnie Lane (milk)	check	6.42	2.13
	10-	6.71	2.42
	50-	8.04	1.08
	100-	26.00	1.04
Mt. Rose (syrup)	check	11.71	1.75
	10-	5.33	0.29
	50-	8.58	0.13
	100-	2.67	0.53

In a separate area from the test site, a virus known from laboratory rearings to be present in a latent form, was beginning to show an increased effect naturally.

Evaluation of Virus Establishment

In October 1959, egg masses were collected from sprayed bushes in each of the treated blocks. These larvae hatched from the eggs were reared in aseptic conditions to determine what, if any, effect the virus spray would have on the 1960 tussock moth brood. This test showed that larvae reared from egg masses collected where virus was present, either naturally or applied, were affected by virus. Unfortunately, it was not possible to determine if the virus was transmitted transovarially or through contact with virus present in the hairy covering of the mass. Nevertheless, the rearing indicated that the 1960 brood within the test plots would be affected by virus. Figure 1 presents the results of two replications of rearing tests in the laboratory. Larvae were reared from lots of eggs that were pooled from (1) plots where syrup was used as sticker, (2) plots where milk was the sticker, and (3) plot where native virus was found. It is interesting that mortality from native virus peaked in the 4th instar, whereas the syrup (Mt. Rose) dosages caused peak mortality to occur in the 5th larval instar. The mortality curve for the dosages using powdered milk as a sticker (Winnie Lane), shows a brood peak extending through the 4th, 5th, and 6th instars.

The Mt. Rose test area, when examined in June 1960, showed a marked reduction in tussock moth population and while there was evidence of the presence of virus within the test plots, population levels were too low to accurately assess the effect of virus on the 1960 brood. Just beyond the edge of the treated area, the larval population was estimated to be nearly ten times as great as in the test area. The line between low and high larval population densities was distinct and, for the first 10 to 20 feet within the high population zone, many of the tussock moth larvae had been killed by virus. Beyond 20 feet from the edge, within the high population zone, the number of virused larvae decreased rapidly. This indicates the virus is spreading slowly from the inoculation area and shows, as was expected, the virus is more effective where the pest population is high. The spread of virus may have occurred through wind dissemination. This spread of virus encroached upon the check plots and each test plot had been influenced by the other. Therefore, a reliable comparison between check and sprayed plots could not be made a year after the test.

The Winnie Lane test area was examined early in July 1960. A sample of seventy-two 15-inch twigs revealed only 1 live pupa within the treated plots. The population has been effectively reduced by virus but, as in the Mt. Rose area, it was impossible to determine percent reduction of the 1960 brood due to virus. Unfortunately, the test site was in the process of being cleared for a housing subdivision so no evaluation of spread of virus or residual tussock moth population adjoining the plots could be made.

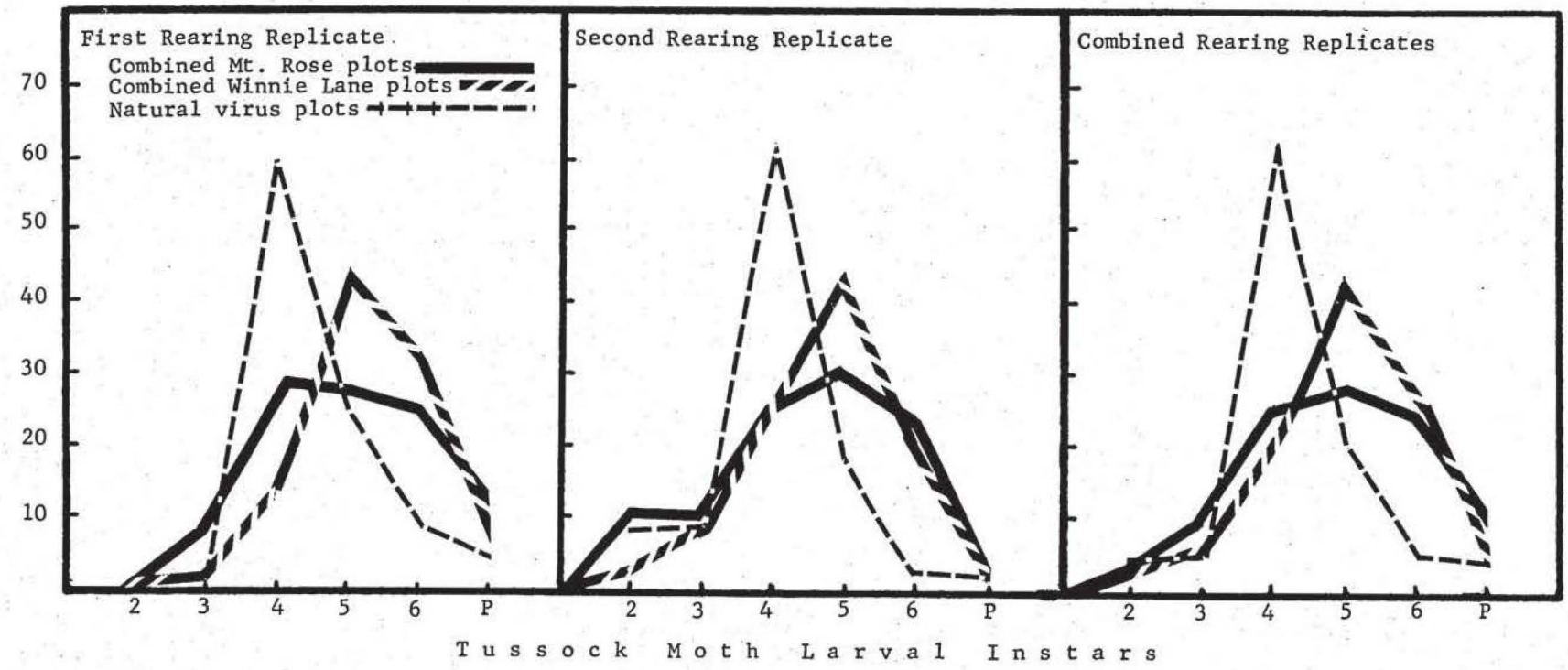

Figure 1.--Mortality curves showing results of laboratory rearing of egg masses collected in October 1959 from field test plots.

DISCUSSION

While tussock moths can be controlled by virus disease, several important factors needed to be determined before virus sprays could be used effectively in field applications to control tussock moths.

This study attempted to determine susceptibility of various tussock moths to the Douglas-fir tussock moth virus, approximate dosage required for establishment, and the expected mortality curve from the various dosages tested. In addition, it was necessary to develop practical means of propagating virus so that an adequate supply would be available for field tests.

Although some difficulty was encountered in laboratory rearings, particularly where both aseptic and infected larvae were reared in the same room, this work showed that tussock moths infesting bitterbrush in Nevada, white fir in New Mexico, and of course Douglas-fir in southern Idaho were susceptible to the virus stock.

Laboratory rearings provided a range of virus concentrations that caused establishment in the rearing cages. Since the range of influencing variables, such as temperature, humidity, confinement of host insects, could not be controlled under field conditions, it was decided to use the most satisfactory laboratory concentration as the minimum dosages used in the field. This, we felt, would insure virus establishment for at least one of the dosages and would provide information upon which later tests could be designed to determine dosage requirements necessary for a given amount of first year mortality.

We were successful in establishing the virus in the bitterbrush field tests and can show a significant mortality from virus for four of the six plots. However, two of the treated plots did not show a significant mortality above the check plots. The reason the 10 million powdered milk concentrations did not show a significant mortality cannot be explained satisfactorily. Our feeling is that powdered milk is not as good a sticking agent as corn syrup, and the 10 million concentration borders on the minimum dosage required to obtain significant mortality in the population density sprayed.

The prespray population density in the 100 million corn syrup plot was the lowest of any plot. In fact this plot had less than half the population density found in any other plot. The population density averaged only 2.67 larvae per 15-inch twig and could have been less than the density necessary for virus establishment in this test. Literature has pointed out that viruses are density-dependent factors. This could explain why the 100 million corn syrup formulation did not show a significant mortality.

It is interesting to note that noticeable virus spread did not go more than 20 feet beyond the treated areas one year after application. The spread in the native virus area was comparable to the test areas in that spread was gradual and no new spots of virus activity was noted in the immediate vicinity.

A critique of this study points out several areas where future tests of this nature could be improved. Obviously, if a future test was designed to follow virus establishment and subsequent mortality, and dissemination for more than one generation, the plots should be separated by more than the 2-chain distance used in this study. The distance should be sufficient to prevent contamination between plots as the virus spreads beyond the plots. Whenever possible, the population density of the pest and host plant should be comparable for all plots. The pest density should also be sufficicient to permit virus establishment. Before an area is chosen for an establishment test it is essential to check for and evaluate the potential of any latent virus.

As with other studies, this work points to the need for future work. For example, a large-scale pilot control test is needed to determine if satisfactory establishment and mortality can be obtained under control conditions. Properly designed, a pilot control test should provide opportunity to determine establishment against controlled pest population density by use of caged samples, as well as against variation of natural population densities. Additional work is needed to determine minimum host pest density to obtain establishment, to determine median lethal dosages (LD50's) on pest densities and on various host plants.

Should the opportunity arise, we plan to conduct a pilot control test which will enable us to gather information of some of these unknowns.